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(54) **Identification of Streptococci**

(57) A process for the rapid identification of Streptococcus bacteria comprises the culture of said bacteria in a medium stimulating the production of certain characteristic enzymes, introducing into samples of the culture reagents each giving a separate colour product of reaction with one of said enzymes and identifying the enzymes by colour produced.

GB 2 005 410 A

SPECIFICATION

Process of rapid identification f bacteria f th genus Streptoc ccus

The present invention relates to a process of rapid identification of bacteria of the genus streptococcus.

In bacteria belonging to the genus Streptococcus there are found species that are very different in their bacteriological criteria. Thus, the method of identification now in use make it possible, by resorting to seriological techniques to classify Streptococci into different serogroups designated by the letters A to H and K to T, the groups that have been found to be responsible for infections in man or animal being, in order of importance, the following: A, D, B, C, G, H, F and K.

There are already methods of identification of these bacteria which use their nutritional characteristics.

In these methods, two series of tests are applied:

-determination of the growing capacity of the bacteria in "hostile" media, the bacteria being inoculated in one or more culture media whose physical and chemical characteristics are made unusual, either by addition of inhibitors, by keeping the pH conditions very basic or by keeping the incubation temperatures abnormally low or high.

-determination of the ability to use natural carbon substrates in a complete culture medium: assimilation of simple sugars.

These identification methods have always had the drawback of requiring long implemention, which can exceed 48 hours, and their empirical nature makes them inexact.

The present invention is intended to make possible the rapid identification of bacteria of the genus Streptococcus. According to the present invention there is provided a process of identification of bacteria of the genus Streptococcus utilising colorimetric display of characteristic enzymes of said bacteria after the culture of the said bacteria in a medium stimulating the production of said enzymes.

Systematic study of numerous classes of substrates has made it possible to select certain families, known in themselves, but which have never been applied to identification of Streptococci or which have been used in too partial a manner to make it possible to detect their discriminating advantages.

According to a first preferable process of the invention, Streptococci belonging to groups A and D have an enzyme of the arylamidase class which can be characterized by hydrolysis of the molecule of pyrrolydonyl-β-naphthylamide or the corresponding nitroanilide, pyrrolydonyl nitroanilide.

Streptococci belonging to groups C and G have an enzyme of the osidase class and more precisely of the glycuzonidase type may advantageously be characterized by hydrolysis of naphthol ASBI β D glucuronic acid or of the corresponding methylumbelliferyl derivative, methylumbelliferyl β D glucuronic acid.

Streptococci belonging to group D have two different enzymes, both of the osidase class. According to a further preferred process of the invention, the enzyme of Streptococci belonging to group D, which is of the glucosaminidase type, may be characterised by hydrolysis of naphthyl-N-acetyl β D glucosamine or the corresponding nitrophenyl or methylumbelliferyl derivative, such as nitrophenyl-N-acetyl β-D-glycosamine of methylumbellieferyl-N-acetyl-β-D glucosamine.

The enzyme of Streptococci of group D, which is the β glucosidase type, may be advantageously characterized by hydrolysis of 6-bromo-2-naphthyl-β-D glucopyranoside or the corresponding nitrophenyl or methylumbelliferyl derivative, such as nitrophenyl β-D glucopyranoside or methylumbelliferyl β-D glucopyranoside.

Streptococci belonging to groups A, B, D and G have an enzyme of the phosphatase class and which, according to the invention, may be characterized by hydrolysis of 2-naphthyl phosphate or the corresponding nitrophenyl derivative, such as nitrophenyl phosphate.

In the process of rapid identification of bacteria of the genus Streptococcus according to the invention, display of the above enzymes, taken together or separately, may be performed after putting in contact with a bacterial culture of Streptococci made in a medium whose composition permits the making, by the bacteria, of an inducible enzymatic equipment.

The influence of this culture medium is therefore determining because the response of a given substrate, in the presence of the same bacterium, will be different depending on the medium on which this bacterium is grown.

Actually it is known that bacteria have a constitutive enzymatic equipment i.e. they are capable of performing a certain number of reactions necessary for their growth.

But bacteria can also have an inducible enzymatic equipment; this means that in the absence of the usual nutriments or in the presence of an insufficient of these nutriments (such as glucose) and in the presence of unusual nutriments (such as a β-glucoside), the bacterium can manufacture the enzyme or enzymes (here β-gludosidase)necessary for degradation of the unusual nutriment into the usual nutriment. In the pr sent case, β-glucoside is transformed into glucose under the action of β-glucosidase. This usual nutriment then participates in the development of the bacterium by means of the constitutive enzymes.

Finally, the bacteria can have their inducible enzymatic equipment repressed; this means that in case th bacterium is simultaneously in the presence f sufficient amounts of usual and unusual nutriments, manufacture of th enzym or enzymes necessary for degradation of the unusual d gradation is prevented or repressed by the presence of the usual nutriment.

The applicant has found that showing the characteristic enzymes of bacteria of th genus Streptococcus,

according to the invention, is optimal when the glucose content of the culture medium is less than or equal to 1 g per litre.

EXAMPLE

A typical composition of the culture medium is given below:

Casein trypsin peptone	8.2 g
Soya papain peptone	1.0 g
Sodium choride	1.7 g
Monopotassium phosphate	0.83 g
Meat peptic peptone	2.5 g
Yeast extract	5.0 g
Tris (hydroxymethyl) aminomethane	3.0 g
Hemin	0.01 g
Cystine	0.4 g
Glucose	1.0 g
Distilled Water (enough for)	1000 ml

the pH being kept at 7.6± 0.2.

Practice of the process according to the invention is advantageously performed on the support described in French Patent No. 76 05 165 of the applicant.

This support is made up of a certain number of discs prepared from a layer of fibres that are chemically inert and insoluble in current organic solvents, these fibres having a loose and sufficiently fine texture to permit absorption and uniform distribution of the reagents over their entire surface, the layer being impregnated with an alcohol solution of a substrate and a pH stabilizer very soluble in water.

The discs are placed on the bottom of a corresponding number of cupules made in a plate made from a material that is inalterable under test conditions. The process of rapid identification of bacteria according to the invention consists in cultivating the bacterium to be identified in the culture medium of the composition mentioned above, or a similar composition, so that said bacterium manufactures an inducible enzymatic equipment until a dense culture is obtained (5 to 24 hours).

The culture is then distributed in a certain number of cupules containing the supporting discs impregnated with various substrates. They are allowed to incubate for 2 to 5 hours at a temperature on the order of 37°C, then there is deposited on each of the supports a reagent able to give a coloured product of reaction with the enzymatic degradation product or products of the substrate, in case the degradation product of the substrate is not itself coloured.

The colours observed are then used to classify the bacterium in one of the Streptococcus groups (A/pyogenes, B/agalactiae, C, D, G, etc.).

Thus, for example, under the action of a β-glucosidase made by the bacteria, 6-bromo-2-naphthyl β∂ D-glucopyranoside is split into 6-bromo-2-naphthol and glucopyranoside, colourless compounds; addition to a diazonium salt, such as fast blue BB gives a violet colouring by coupling with the naphthol.

Also, under the action of a glycylglycylaryl-amidase, the glycylglycyl-β-naphthylamide is split into glycylglycine and 2-naphthylamine, the coupling of this latter compound with the diazonium salt mentioned above leading to the formation of an orange colouration.

Under the action of a phosphatase, 2-naphthylphosphate is split into phosphonic acid and 2-naphthol whose coupling with fast blue gives a violet colouration.

It is understood that these few examples of colourimetric reaction have been given only by way of indication and in no way limit the scope of the invention claimed hereinafter.

CLAIMS

1. A process of identification of bacteria of the genus Streptococcus, utilising colorimetric display of characteristic enzymes of said bacteria after the culture of the said bacteria in a medium stimulating the production of said enzymes.

2. A process according to claim 1, in which the medium stimulating the production of the enzymes contains at a maximum 1 gram per litre of glucose.

3. A process according to claim 1 or claim 2, in which the characterization of Streptococci A and D is performed thanks to the showing of an enzyme of the arylamidase class, by hydrolytic splitting of pyrrolydonyl β napthylamide or the corresponding nitroanilide derivativ , pyrrolydonylnit-roanilide.

4. A process according to claim 1 or claim 2, in which the characterization of the Streptococci C and G is performed thanks to showing an enzyme of the osidas type and more particularly of th glucuzonidase type, by hydrolytic splitting of naphthol β D glucuronic acid or the corresponding methyl umbelliferyl derivative, methylumbelliferyl β Dglucuronic acid.

5. A proc ss according to claim 1 or claim 2, in which the characterization of Streptococcus D is performed thanks to showing an enzyme of the osidase class and more particularly of the glucosamidinas type, by hydrolytic splitting of naphthyl N acetyl β D glucosamine or the corresponding nitro-phenyl or methyl umbelliferyl derivatives, such as nitrophenyl-N-acetyl-β-D-glucosamine or methyl umbelliferyl-N-

acetyl-β-D-glucosamine.

6. A process according to claim 1 or claim 2 in which the characterization of Streptococcus D is performed thanks to showing an enzyme of the osidase class and more particularly of the βglucosidase type, by hydrolytic splitting of 6-bromo-2-naphthyl-β-D-glucopyranoside or the corresponding nitro-phenyl or methyl umbelliferyl derivatives, such as nitro-phenyl-β-D-glucopyranoside or methyl umbelliferyl-β-D-gluco-pyranoside.

7. A process according to claim 1 or claim 2, in which characterization of Streptococci A, B, C and G is performed thanks to showing of an enzyme of the phosphatase class, by hydrolytic splitting of 2-naphthylophosphate or the corresponding nitro-phenyl derivative, nitro-phenylphosphate.

8. A process according to any one of claims 1 to 7, in which the showing of the characteristic enzymes of bacteria of the genus Streptococcus is performed separately or simultaneously on a device consolidating all the elements necessary for performance of the tests.

9. A process as claimed in claim 1 substantially as hereinbefore described.

10. Any novel subject matter or combination including novel subject matter herein disclosed, whether or not within the scope of or relating to the same invention as any of the preceding claims.

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